REMARKS

The Official Action dated February 11, 2005 has been carefully considered. Accordingly, the amendments presented herewith, taken with the following remarks, are believed sufficient to place the present application in condition for allowance. Reconsideration is respectfully requested.

By the present Amendment, claims 1-2, 8, 15 and 20 have been cancelled. Claims 3, 12 and 22 are amended to clarify the limitations therein. Claims 3, 5, 6, 11-14, 16 and 18-19 are amended as to matters of form. It is believed these changes do not involve any introduction of new matter, whereby entry is believed to be in order and is respectfully requested.

Claims 5, 15 and 22 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The Examiner asserted, pursuant to the suggestion of the Board, that the metes and bounds of the claims are unclear with the recitation: "pH is equal to or lower than pH 7". Specifically, the Examiner asserted that it is unclear to which process step the claimed pH limitations would meet. This rejection is traversed. However, to expedite prosecution of the application, claims 5 and 22 have been amended to recite that the pH "of the cells prior to the addition of the metal salt" is equal to or lower than pH 7. Because claim 15 has been cancelled, the rejection to claim 15 is moot. Applicants submit that amended claims 5 and 22 are definite and that the rejection of the claims under 35 U.S.C. §112, second paragraph, has been overcome. Reconsideration is respectfully requested.

Claims 5, 8, 12, 15, 18-19 and 21-22 were rejected as being obvious over Builder et al (U.S. Patent No. 5,663,304). The Examiner asserted that Builder et al teach a method for the production of recombinant peptides comprising fermenting cells to produce recombinant peptides in the presence of metal salt prior to peptide isolation. Specifically, with regard to claims 5, 15 and 22, the Examiner asserted that Builder et al teach that an alkaline metal salt buffer was added after fermentation and the pH was adjusted to 3.5 with phosphoric acid. With regard to claims 8, 12, 18-19 and 21, the Examiner asserted that Builder et al teach that the method includes human growth hormone as a preferred mammalian polypeptide. Accordingly, the Examiner asserted that it would have been prima-facie obvious to modify or

substitute the method of producing a recombinant polypeptide as taught by Builder et al with the step of producing a recombinant human growth hormone to achieve the expected advantage of developing a method for manufacturing human growth hormone.

However, as will be set forth in detail below, Applicants submit that the methods recited in claims 5, 8, 12, 15, 18-19 and 21-22 are nonobvious over and patentably distinguishable from the teachings of Builder et al. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

More particularly, claim 21 recites a method for the reduction of the amount of trisulfides in the production of recombinant growth hormone. The method comprises fermenting cells to produce recombinant growth hormone. A metal salt is added during or after the fermentation step, prior to growth hormone isolation.

Claim 22 recites a method for the reduction of the amount of trisulfides in the production of recombinant peptides. The method comprises fermenting cells to produce recombinant peptides. A metal salt is added during or after the fermentation step prior to peptide isolation. The pH of the cells prior to the addition of the metal salt during and after fermentation is less than or equal to 7.

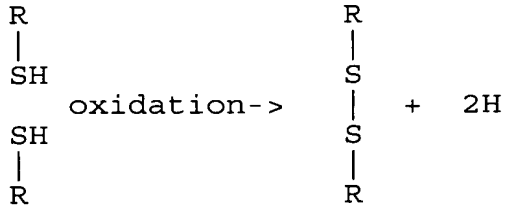
Applicants find no teaching, suggestion or reference in Builder et al of the claimed methods. In fact, Builder et al are silent as to the problem of trisulfide formation in recombinant peptide production let alone a solution to the problem.

Builder et al is directed to a method of refolding (isolating) proteins from host cells, specifically IGF-1, in the presence of a complex composition (*"special buffer")* having a pH between 7-12 comprising 5-40% (v/v) of an alcoholic or polar aprotic solvent, alkaline earth, alkali metal or ammonium salt, a chaotropic agent, and 0.01 to 15 μM copper or manganese salt (column 6, line 42 - column 7, line 21). Builder et al disclose at column 7, lines 10-12 that: *"the essence of the invention is in utilizing a special buffer containing a minimal concentration of copper or manganese salt to enhance refolding of misfolded polypeptides."* Builder et al also disclose that the alcoholic or polar aprotic solvent, alkaline earth, alkali metal or ammonium salt, chaotropic agent, and copper or manganese salt are all "key ingredients" (column 12 lines 6 – 54).

The presently claimed invention does not involve a method of refolding proteins using the "special" buffer of Builder et al containing the "key ingredients" alcohol and chaitropic agent. The present methods are directed to methods for the reduction of the amount of trisulfides in the production of recombinant peptides comprising fermenting cells to produce recombinant peptides, wherein a metal salt is added during or after fermentation prior to peptide isolation. Accordingly, as Builder et al is directed to methods for refolding mis-folded polypeptides using special key ingredients, Applicants find no teaching, suggestion or reference of the present methods utilization of metal salts during or after the fermentation step prior to isolation of the recombinant peptides to solve the problem of trisulfide formation.

The Examiner asserted that Builder et al teach that metals facilitate disulfide oxidation in polypeptides and yield correct refolding of misfolded polypeptides contained in host cells. However, once again the problem of facilitating disulfide oxidation during refolding is not the same as reducing trisulfilde formation during production, prior to isolation.

A disulfide bond (SS-bond), also called a disulfide bridge, is a strong covalent bond between two sulfhydryl groups. This bond is very important to the folding, structure, and function of proteins. When two amino acids bond to each other through their side chains, they normally do so through a disulfide bond. The particular side chain involved is the sulfhydryl group (-SH). Oxidation of the thiol group yields a disulfide (S-S) bond. The presence of S-S then helps to maintain the tertiary structure of the protein. An amino acid that commonly forms S-S bonds in proteins is cysteine. When two cysteines are bonded by an S-S bond, the resulting molecule between the two protein chains is called cystine. The figure below shows the formation of a disulfide bond. The R on each side group represents the remainder of the amino acid.



Builder et al, teach that metal salts, specifically of copper and manganese (transition metals), facilitate thiol oxidation to form a disulfide bond. This teaching when extended, would suggest that if metal salts facilitate disulfide bond formation, metal salts would also facilitate trisulfide bond formation. Therefore, as the present invention is directed towards methods for the reduction of the amount of trisulfide in the production of recombinant peptides, Builder et al teach away from the present invention. As a "useful general rule", references that teach away cannot serve to create a prima facie case of obviousness, *In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994). Accordingly, the present invention, which solves the problem of trisulfide formation in peptides, is non-obvious from the teachings of Builder et al.

The Examiner also asserted that Builder et al teach a method of producing polypeptides in the presence of an alkali metal salt prior to isolation. To support this assertion the Examiner refers to the fermentation exemplified in the specification where an alkali metal salt is a component of the culture medium (column 23, line 26 - column 27, line 30). However, Builder et al disclose that: "*production of IGF-1 occurred after the phosphate in the medium was depleted*". (column 27, lines 25-26) The source of the phosphate is the alkali metal salt. If the phosphate is being depleted prior to the production of the polypeptide, there can be no teaching or suggestion by Builder et al of the presently claimed methods because the present methods employ metal salt during or after fermentation to produce recombinant peptides with a reduction in the amount of trisulfides.

The Examiner further asserted that Builder et al teach that an alkali salt buffer (pH 10.5) was added after fermentation and the pH was adjusted to 3.5. However, since this adjustment in Builder et al is made during isolation (refolding), this disclosure is irrelevant because the presently claimed methods recite that the pH of the cells, prior to the addition of the metal salt, during and after fermentation is less than or equal to 7.

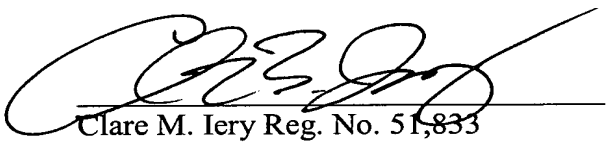
To establish prima facie obviousness of the claimed invention, all of the claim limitations must be taught or suggested by the prior art, *In re Royka*, 180 U.S.P.Q. 580 (C.C.P.A. 1974). Furthermore, references relied upon to support a rejection under 35 U.S.C. §103 must provide an enabling disclosure, i.e., they must place the claimed invention in the possession of the public, *In re Payne,* 203 U.S.P.Q. 245 (C.C.P.A. 1979). Not only do Applicants find no teaching, suggestion or reference by Builder et al of the methods as

defined by the claims, Applicants find no teaching, suggestion or reference in Builder et al for modifying the disclosures therein to arrive at the claimed invention. In view of the failure of Builder et al to teach, suggest or recognize the methods as defined by the claims, the reference does not provide an enabling disclosure of the present invention and therefore does not support a rejection of claims 5, 8, 12, 15, 18-19 and 21-22 under 35 U.S.C. §103.

It is therefore submitted that the methods recited in claims 5, 8, 12, 15, 18-19 and 21-22 are nonobvious over and patentably distinguishable from the teachings of Builder et al, whereby the rejection under 35 U.S.C. §103 has been overcome. Reconsideration is respectfully requested.

It is believed the above represents a complete response to the rejections of the claims under 35 U.S.C. §§103 and 112, second paragraph, and places the present application in condition for allowance. Reconsideration and an allowance are requested.

Respectfully submitted,



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